

1

2

3

4

5

6 Microbial C, N and P in soils of Mediterranean oak forests:

7 influence of season, canopy cover and soil depth

8

9 Cristina Aponte, Teodoro Mara  n, Luis V. Garc  a

10

• Running head: Microbial C, N and P in soils of Mediterranean oak forests

• Article type: General research

• Title: Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth

• Authors: Cristina Aponte¹, Teodoro Marañón¹, Luis V. García¹

• Affiliation: ¹Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNASE) – CSIC P.O. Box 1052, 41080 Sevilla, Spain.

• Author for correspondence: Cristina Aponte, Tel: +34 95 4624711, Fax: +34 95 4624002, email: aponte@irnase.csic.es

Key words

Microbial biomass, nitrogen, nutrient immobilization, phosphorus, plant-soil interactions, seasonal dynamics, vegetation cover

Abstract

In Mediterranean ecosystems the effect of aboveground and belowground environmental factors on soil microbial biomass and nutrient immobilization-release cycles may be conditioned by the distinctive seasonal pattern of the Mediterranean-type climates. We studied the effects of season, canopy cover and soil depth on microbial C, N and P in soils of two Mediterranean forests using the fumigation-extraction procedure. Average microbial values recorded were $820 \mu\text{g C g}^{-1}$, $115 \mu\text{g N g}^{-1}$ and $19 \mu\text{g P g}^{-1}$, which accounted for 2.7%, 4.7% and 8.8% of the total pools in the surface soil, respectively. Microbial N and P pools were about 10 times higher than the inorganic N and P fractions available for plants. Microbial C values differed between forest sites but in each site they were similar across seasons. Both microbial and inorganic N and P showed maximum values in spring and minimum values in summer, which were positively correlated with soil moisture. Significant differences in soil microbial properties among canopy cover types were observed in the surface soil but only under favourable environmental conditions (spring) and not during summer. Soil depth affected microbial contents which decreased twofold from surface to subsurface soil. Microbial nutrient ratios (C/N, C/P and N/P) varied with seasons and soil depth. Soil moisture regime, which was intimately related to seasonality, emerged as a potential key factor for microbial biomass growth in the studied forests.

Our research shows that under a Mediterranean-type climate the interaction among season, vegetation type and structure and soil properties affect microbial nutrient immobilization and thus could influence the biogeochemical cycles of C, N and P in Mediterranean forest ecosystems.

Introduction

Soil microbes play an essential role in the main biogeochemical transformations of organic matter and in soil fertility (Jenkinson & Ladd 1981). During the mineralization process, an important fraction of the C, N and P in the decomposing residues is immobilized in the microbial biomass as part of their cellular constituents (e.g. phospholipids and proteins), and then released upon microorganism death (Anderson & Domsch 1980; Jonasson et al. 1999). The capacity of microorganisms to act both as a sink and a source of nutrient resources is particularly relevant for plant nutrition since most of the annual N and P requirements of land plants are supplied from the decomposition of organic matter in the soil (Singh et al. 1989). Changes in biomass, physiology, composition and activity of soil microbes may affect their functional capacity and thus the ecosystem geochemical processes (Balser & Firestone 2005; Crenshaw et al. 2008).

Both aboveground and belowground factors affect microbial biomass and, therefore, nutrient availability (García et al. 2002; Schade & Hobbie 2005). Vegetation structure and composition exert a control on microbial growth through litter quality and quantity and root exudates that determine the input fluxes of labile C and nutrients (N, P) (Fisk & Fahey 2001; Kara et al. 2008). Soil chemical and physical characteristics, like soil organic matter and soil structure and texture, may also constrain microbial developments (Hassink 1994), and the variability of these properties along soil profile is reflected in the microbial communities (Fierer et al. 2003). Climatic conditions have a direct effect on microbial communities through soil moisture and temperature (Ley et al. 2004; Nielsen et al. 2009), but they may also have an indirect effect through interactions with other factors such as vegetation, topography and landscape (Malchair & Carnol 2009; Myers et al. 2001). Temporal patterns of microbial growth and nutrient

immobilization-release cycles usually reflect seasonal changes (but see Raubuch & Joergensen 2002) although such a response vary among ecosystems depending on their particular moisture and temperature regimes (Bohlen et al. 2001; Ley et al. 2004; Nielsen et al. 2009).

In this study we investigated the effects of several abiotic and biotic factors on the soil microbial C, N and P in Mediterranean oak forests. Mediterranean ecosystems are subjected to a marked seasonality that imposes a severe summer drought after a favourable rainy autumn and spring, that is reflected in soil microbial dynamics (Quilchano & Marañón 2002). Vegetation traits that overcome Mediterranean summer drought, such as long-lived and hard leaves (sclerophylly), influence litter quality and quantity and thus decomposition processes (Gallardo & Merino 1993; Rutigliano et al. 2004). In addition Mediterranean forests have large environmental heterogeneity (species composition, canopy structure, soil properties) that might affect microbial spatial patterns (Joffre et al. 1996; Quilchano et al. 2008). The interplay of driving factors on the microbial dynamics - climate, vegetation and soil - is key to understanding the biogeochemical cycles in Mediterranean forests (Bohlen et al. 2001); however there are few studies on microbial biomass and nutrient dynamics in this type of ecosystem (Gallardo et al. 2009).

Mediterranean ecosystems, as other areas under semiarid climate conditions, are predicted to experience warmer and drier conditions due to climate change (Bates et al. 2008). Information on how microbial nutrients immobilization-release cycles are affected by environmental factors under the characteristic Mediterranean seasonal pattern could increase our understanding on how climate change may affect microbial controls over nutrient availability in this and other ecosystems and regions.

Our main objective was to investigate the main factors affecting microbial C, N and P content in Mediterranean forest soils. In particular we tested the following hypotheses: i) under Mediterranean climatic conditions there are seasonal patterns with higher microbial growth and nutrient retention during the warm and wet season (spring), and a decline in microbial population and nutrient retention during the hot and dry season (summer); ii) distinct vegetation cover and composition affect soil microbial properties, with higher microbial carbon under deciduous trees which have a nutrient richer litterfall; and iii) microbial nutrient content is higher in surface than in the subsurface soil. We also tested whether these soil microbial patterns are consistent in different forest sites, and analysed the interactions between these factors.

Methods

Site description

This study was conducted in the oak forests of Aljibe Mountains, near the Strait of Gibraltar, in southern Spain (Figure 1). Acidic, nutrient-poor soils (*Palexeralfs*; Soil Survey Staff, 2006) are developed over Oligo-Miocene sandstone bedrock that is frequently interspersed with layers of marl sediments yielding soils richer in clay (*Haploxererts*; Soil Survey Staff, 2006). Climate is subhumid Mediterranean-type with warm, dry summers, and humid, mild winters. Temperatures average 24°C in summer and 8.5°C in winter. Mean annual rainfall varies from 701mm to 1331mm, depending on the mountain topography, and most (95%) of it falls from October to May. Vegetation is dominated by sclerophyllous evergreen cork oak (*Quercus suber* L.), mixed with the winter-deciduous Algerian oak (*Q. canariensis* Willd.) which is locally abundant in the valley bottoms (Urbieto et al. 2008). Both oak species differ in their leaf fall and litter quality: *Q. canariensis* has a higher nutrient content (Ca, K, Mg, S) and a

lower C/N ratio compared *Q. suber*, what induces distinct soil conditions via litter decomposition (Aponte et al. in press). There is a diverse shrubby and arborescent understorey of *Phillyrea latifolia*, *Erica* spp. and *Pistacia lentiscus*. See detailed description in Ojeda *et al.* (2000).

For this study two forest stands of different structure, 40 km apart, were selected within the study area. The first one, at San Carlos del Tiradero (36° 9' 46'' N 5° 35' 39'' W) hereafter called "Forest", was located in the south of the study area close to the coast, at 335–360 m a.s.l. on a NE slope. The second stand hereafter called "Woodland" was located at La Saucedá (36°31'54''N 5°34'29''W) and stood inland, in the north of the area, at 530–560 m a.s.l. on a NW slope. The Forest site had a higher density of trees and a close canopy cover while the Woodland site had higher canopy heterogeneity and fewer trees mixed with abundant shrubs and gaps (Table 1). Both sites presented a large heterogeneity in their chemical and physical soil characteristics; see details on the forest sites in Quilchano *et al.* (2008) and Pérez- Ramos *et al.* (2008).

Field sampling

Soil samples were taken in spring (May-June), summer (September) and autumn (December) 2007, and spring (May) 2008. Soils cores were extracted at two depths (0-8 cm and 8-16 cm) using an auger. Each sample was composed of four subsamples. At the Woodland site four microhabitats corresponding to different vegetation cover types were studied i.e. soil beneath the canopy of *Q. canariensis* (Qc), beneath *Q. suber* (Qs), under shrubby cover (S) and in gaps with grass cover (G). At the Forest site, soils beneath two types of canopy cover - *Q. canariensis* and *Q. suber* - were studied. To minimize the effect of the inherent site variability ten replicates of each microhabitat were sampled at each season and soil depth, which made up a total of 480 samples. Two

30x30cm quadrates were used to assess the thickness of the litter layer, using a folding rule; and the litter biomass, by the harvesting and drying method (expressed as kg dry mass m⁻²) at each sampling point. This sampling design allowed us to assess the effects of three factors: season, vegetation cover type and soil depth on microbial C, N and P, and to test their consistency between the two sites.

Laboratory analysis

Soil samples were brought to the laboratory in an ice-box and they were stored at 4°C for a maximum of three days. Stones, roots and other recognizable plant parts contained in the samples were removed and the soil was homogenised through a 2mm sieve. A subsample of 1g was used to determine the water content gravimetrically by weighing the fresh and dried (105°C) soil. The same subsample was then incinerated for 4 hours at 550°C to determine the soil organic matter content by calcination method (Sparks 1996).

Microbial C, N and P were estimated in the fresh soils using a chloroform fumigation-extraction procedure (Brookes et al. 1985; Brookes et al. 1982; Vance et al. 1987). Two soil subsamples (10g and 5g) were extracted using 50ml of 0.5M K₂SO₄ or 50 ml of 0.025N HCL + 0.03N NH₄F for subsequent determination of microbial C and N or microbial P, respectively. The other two soil subsamples (10g and 5g) were fumigated with chloroform for 24h in a vacuum desiccator, followed by the same extraction procedure as the unfumigated samples. The soil extracts were frozen until their C, N, P content were measured. The C and N in the fumigated and unfumigated soil extracts were determined using a Total Dissolved Organic Carbon and Nitrogen Analyzer (TOC-Vesh). Microbial C and N were estimated as the difference in K₂SO₄-extractable dissolved organic carbon or nitrogen between fumigated and unfumigated

soils using as extractability correction factors: $K_c=0.45$ for C and $K_n= 0.40$ for N (Jonasson et al. 1996; Rinnan et al. 2008). Available P in NH_4F soil extracts was measured using the Bray Kurtz method (Bray & Kurtz 1945). Microbial P was estimated as the difference in available P between the fumigated and the unfumigated soil using a correction factor $K_p= 0.40$ (Brookes et al. 1982).

Soil ammonium and nitrate and total C, N and P contents were analysed in the unfumigated soils. Inorganic nitrogen (NH_4^+ and NO_3^-) was extracted using 2M KCL and determined by distillation–titration in a Bran-Luebre Autoanalyzer. Soil total C and N were estimated using an Autoanalyzer LECO. Soil total P was determined by acid digestion and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) analysis (Sparks 1996). Concentrations of the elements are given on a dry weight basis ($105^\circ C$).

Data Analysis

To evaluate the effects of seasonality and canopy cover type on the soil microbial properties of the two forest sites at the two soil depths we used repeated-measures analysis of variance (ANOVA) on a split-plot design with sampling season defined as a within-factor of four levels, each one divided into two forest types (large units in the split-plot design) and each one further divided into canopy cover types (split-plots). Because of the unbalanced design, we first run the analysis including only the common cover types (*Q. canariensis* and *Q. suber*) of the two forest sites, and then analysed the differences between the microhabitats within each site. Variables were transformed (log, arcsine) to meet necessary assumptions of normality and homocedasticity. Post-hoc comparisons were done using Fisher LSD test and type I error inflation resulted from repeated tests was controlled using the False Discovery Rate (García 2003). This

technique was preferred over Bonferroni-related procedures that notably increase power losses. General trends of soil microbial C, N and P values were related to other soil features (moisture, organic matter, inorganic nutrients) by correlation analysis.

Results

Soil patterns

There were significant differences in soil properties between canopy cover type and soil depth, and also between sites (Appendix 1). Soil water content varied across season attaining minimum values in summer (average of 10.9%) and maximum values in spring (average of 19.2%) (Figure 2). Moisture decreased with soil depth across all studied soils ($F= 36.89$, $p<0.0001$). Differences in soil moisture between the forest sites were only found in the spring ($p <0.0001$) when soil in the Woodland site had a higher gravimetric water content than in the Forest. In each site, soil moisture values were similar for all vegetation covers in each season. Soil texture varied significantly between the forest sites with clayey soils in the Woodland ($\approx 30\%$ clay, 49% sand) and sandy soils in the Forest ($\approx 16\%$ clay, 65% sand). These differences affect the water holding capacity and moisture availability of their soils. Thus, under similar water content, sandy soils would proportionally have more water held at available potentials than clayey soils.

Soil organic matter (SOM) estimated as loss on ignition averaged over all samples 10.5% and varied from 0.77% to 24.93% in the Woodland and from 4.11% to 24.03% in the Forest (Appendix 1). Surface soil contained higher SOM (average of 12.7%) than the deeper layer (average of 8.35%) in all studied soils ($F= 71.42$, $p<0.0001$). In both sites higher values of SOM were found in the soils beneath *Q. canariensis* and shrub than in the soils under *Q. suber* and herbaceous cover. Maximum litter biomass values

(1.69 kg m⁻²) were recorded in the Forest. Gaps with grass cover (in the Woodland site) had a significantly lower litter mass (0.09 kg m⁻²) and litter layer thickness (0.56 cm) than any other microsite (p<0.0001).

Soil ammonium averaged 7.3 µg g⁻¹ in the Woodland and 4.0 µg g⁻¹ in the Forest. Similar available phosphorus values were recorded in all soils (average of 2.5 µg g⁻¹). In general the concentration of both nutrients decreased with soil depth (p<0.022) except in the case of ammonium in the Forest (p<0.850).

Soil total C and N ranged from 1.0% to 7.7% and from 0.1% to 0.7% respectively. Both soil total C and N decreased with soil depth (p<0.0001) and total N content varied among soils. The Ct/Nt ratio averaged 14.7 and the ratio increased with soil depth (F=43.75, p<0.0001). The highest Ct/Nt values were recorded in *Q. suber* soils (>15.5) while soils beneath *Q. canariensis* in the Woodland had the lowest carbon to nitrogen ratio (12.9). Total P in the soil ranged from 119 to 484 µg g⁻¹ and average values were higher in the Woodland (312 µg g⁻¹) than in the Forest (259 µg g⁻¹). In the Woodland total P (F=24.941, p<0.0001) varied significantly among canopy cover types, with maximum average values (for two soil depths) beneath *Q. canariensis* (368 µg g⁻¹) and minimum in the grasslands (282 µg g⁻¹).

Microbial C, N and P pools

Microbial C pool (Cm) averaged over all samples 820 µg g⁻¹ and ranged from 121 µg g⁻¹ to 3232 µg g⁻¹ (Table 2). The proportion of microbial C to total soil C averaged 2.2%. Microbial N (Nm) averaged 115 µg g⁻¹ and contributed 4.0 % (range of 0.7%-9.9%) to the total soil nitrogen while inorganic N (NH₄⁺ and NO₃⁻) contribution averaged 0.3%. Microbial P (Pm) in soils was, in average, 19 µg g⁻¹ and, in the surface soil, it comprised about 9% of the total soil P. The proportion of inorganic available P to the

total soil varied from 0.04% to 3.5% (mean of 0.89%). Average microbial nutrient ratios recorded were Cm/Nm: 8.6, Cm/Pm: 78.1 and Nm/Pm: 9.3. Microbial C, N and P values were positively and significantly correlated with soil moisture ($r>0.46$, $p<0.0001$) and soil organic matter ($r>0.62$, $p<0.0001$).

Microbial response to seasonal conditions

Soil microbial biomass did not significantly differ among seasons (Table 3, Figure 3). Microbial carbon pool was significantly higher in the Woodland, where it ranged from $253 \mu\text{g g}^{-1}$ to $3232 \mu\text{g g}^{-1}$ and contributed 2.15 % to total soil C, than in the Forest where it varied from $1201 \mu\text{g g}^{-1}$ to $1772 \mu\text{g g}^{-1}$ and amounted 1.69% of total soil C ($p<0.0001$). This difference was consistent across all seasons.

Microbial N and P pools varied seasonally with maximum values in spring and minimum values in summer (although the seasonal patterns differed between forest sites). The most important seasonal variations were observed for microbial P which values changed twofold from the spring ($\sim 24 \mu\text{g g}^{-1}$) to the summer ($10 \mu\text{g g}^{-1}$). On average, soils in the Woodland had higher microbial N ($126 \mu\text{g g}^{-1}$) and microbial P ($20 \mu\text{g g}^{-1}$) than soils in the Forest (N: $93 \mu\text{g g}^{-1}$, P: $17 \mu\text{g g}^{-1}$) although this pattern was reversed in the autumn when Pm pool was larger in the Forest. Summer Nm and Pm values were similar for both forest sites while the largest differences between sites were recorded during the spring ($p<0.003$), when the Woodland presented the highest values.

Microbial ratios (Cm/Nm; Cm/Pm; Nm/Pm) changed seasonally ($p<0.0001$) and generally differed between the two forest sites (except for the summer), due to their distinct values of Nm and Pm (Figures 3 and 4).

Season also affected the non microbial pools of available nutrients (Figure 5). K_2SO_4 -extractable dissolved organic carbon values increased in summer matching up

with a slight but not significant decrease in Cm, and declined in spring when the microbial C values were higher. K₂SO₄-extractable dissolved organic nitrogen, inorganic N (ammonia and nitrate) and available inorganic P declined in summer and autumn and increased in spring, mirroring the pattern observed for Nm and Pm ($r \approx 0.35$, $p < 0.0001$).

Effect of vegetation cover type on soil microbial biomass

In the Forest site there were no significant differences in microbial C, N, P pools and their ratios between the soils of the two *Quercus* species at any sampling time and soil depth (Figures 3 and 4).

In the Woodland the effect of the vegetation cover type varied across seasons: significant differences among cover types were observed in the spring while similar microbial values were recorded for all vegetation types in summer. The effect of cover type on soil microbial pools was larger in the upper soil layer ($p < 0.002$ for Cm, Nm and Pm) than in the subsurface soil ($p < 0.029$ for Pm). Higher values of microbial C were recorded in *Q. canariensis* and shrub soils compared to those estimated in soils beneath *Q. suber* and the least beneath grass cover in forest gaps, which constantly showed the lowest Cm values and were not affected by seasonality. As occurred with Cm, soils of *Q. canariensis* and shrubs had higher microbial N than soils under *Q. suber* and herbaceous cover. In contrast microbial P was similarly high beneath the shrubs and the two oak trees while minimum values were consistently recorded in the soils under grass cover. The largest changes in Cm/Nm among vegetation cover types were observed in autumn when the ratio decreased from the two *Quercus* species to shrub soils and grass soils. Cm/Pm and Nm/Pm values tended to vary seasonally and the highest values were recorded in summer related to the limited amount of microbial P. Soils beneath grass

cover, in contrast to other cover types, showed no similar Cm/Pm and Nm/Pm values across seasons. .

Microbial properties and soil depth

All microbial C, N and P decreased twofold with soil depth ($p < 0.0001$); in particular carbon from $1106 \mu\text{g g}^{-1}$ in the upper soil to $534 \mu\text{g g}^{-1}$ in the subsurface soil, nitrogen from $158 \mu\text{g g}^{-1}$ to $71 \mu\text{g g}^{-1}$, and phosphorus from $28 \mu\text{g g}^{-1}$ to $10 \mu\text{g g}^{-1}$. Cm/Nm was higher in the subsurface soil, although this difference was only significant in autumn, when values increased from 8.9 to 13.6. Cm/Pm and Nm/Pm ratios were always significantly higher in the subsurface soil (104.2 and 10.3) than in the upper soil (64.0 and 5.2 respectively) ($p < 0.0001$).

Discussion

Soil microbial carbon, nitrogen and phosphorus in Mediterranean forests

Microbial C and N averaged $820 \mu\text{g g}^{-1}$ and $115 \mu\text{g g}^{-1}$ in the studied Mediterranean forest soils. These values fell within the range of Cm and Nm estimates presented by Wardle (1992) for tropical (Cm: $653\text{--}986 \mu\text{g g}^{-1}$; Nm: $65\text{--}100 \mu\text{g g}^{-1}$) and temperate forests (Cm: $736\text{--}877 \mu\text{g g}^{-1}$; Nm: $93 \mu\text{g g}^{-1}$). They were also in accordance with the few examples of Mediterranean forests: Cm range of $975\text{--}1601 \mu\text{g g}^{-1}$ in a pine forest (Hernández et al. 1997) and Nm of $72\text{--}178 \mu\text{g g}^{-1}$ in an oak savanna (Gallardo et al. 2000).

Recently Cleveland & Liptzin (2007) have revealed the existence of a C:N:P Redfield- like ratio for the soil microbial biomass at the global scale (60:7:1) and for forest soils (74:9:1) that is very close to the average microbial ratio that we have found (78:9:1). Microbial stoichiometry relations are partly dependent on the validity of the

microbial C, N and P estimates which, in fumigation procedures, are dependent on the k-factors used (Ross et al. 1996). Our results confirm that microbial biomass stoichiometry is well constrained and considering that the use of laboratory-determined fixed factors may not be accurate for a diverse soil community and could mask differences between soil types and depths, microbial N:P could be cautiously used, in addition to N:P ratios in plants and soils, to estimate nutrient deficiency in terrestrial ecosystems (Cleveland & Liptzin 2007).

The microbial P recorded in the studied soils ($19 \mu\text{g g}^{-1}$) was close to the lowest P_m values recorded by Joergensen (1995) in 38 beech forest soils ($18\text{-}174 \mu\text{g g}^{-1}$). However the proportion of total P immobilised by microorganisms in the surface soil (8.8%) was higher than that of C (2.7%) and N (4.7%) what suggests that microbial biomass may play an important role in regulating plant phosphorous supply in the studied forests (Joergensen et al. 1995; Jonasson et al. 1996). The microbial fraction of the total N and P pool was almost ten times higher than the estimated available inorganic N (NH_4^+ and NO_3^- ; 0.38% of the total N) and available P (0.89% of total P). This could implicate that nutrient availability for plants might be strongly controlled by microbial dynamics: a flush of available N and P for plants could be released after microbial population decline while microbial population growth might be associated with a strong competition for these growth-limiting resources (Lipson et al. 1999; Schmidt et al. 2007).

Seasonal variations of microbial C, N and P

Microbial C, N and P in the studied soils showed distinct seasonal patterns that were reflected in changes in the microbial component ratios. Seasonal variations of soil microbial carbon had been registered in many ecosystems (Bohlen et al. 2001; Díaz-

Raviña et al. 1993; Miller et al. 2009) including Mediterranean and semi-arid zones (Goberna et al. 2007; Mlambo et al. 2007). Seasonality seems to affect microbial populations through changes in soil moisture and temperature, but it may also indirectly modulate substrate availability through plant phenology (Rinnan et al. 2008).

In this Mediterranean-type climate we expected a decline in microbial carbon during the summer as a result of drought stress. However, microbial C showed no significant differences across seasons, although values tended to be lower during the summer time and they were correlated with soil moisture content. Several processes can occur during summer drought that would explain these results. Under low moisture conditions, soil microorganisms may become isolated in a landscape of disconnected water pockets that impedes the diffusion of substrate, limit microbial growth and may cause death by starvation (Killham et al. 1993; Xiang et al. 2008). On the other hand this disconnection also prevents microorganism to be predated by soil fauna (protozoa, amoeba) which activity and mobility are reduced by soil drought (Kuikman et al. 1989).

Drought stress also affects microbial physiology. Low water potential induces microbial dehydration and might eventually cause death. Drought-tolerant microbes can be inherently protected against low moisture (e.g. thicker cell walls of gram-positive bacteria) or have the capacity to adapt to the external low water availability by accumulating osmolites in their cytoplasm) (Schimel et al. 2007). As a result of drought stress up to 30% of carbon resources can be bound in cytoplasmic osmotic protection molecules, what negatively affects microbial activity and population growth (Schimel et al. 1989). In this study microbial C was estimated using the fumigation-extraction technique that recovers only a fraction of the total microbial C, most of which is cytoplasmic, and relates it to the total by an empirical constant (K_c). Changes in the concentration of cytoplasmic C may therefore be a source of error in microbial biomass

measurements (Ross et al. 1996; Schimel et al. 1989). We suggest that in our studied soils a fraction of the total microbial population might have died during summer drought, what together with summer root decay (Joergensen et al. 1994) could account for the K₂SO₄-extractable DOC peak recorded in this season. The estimated C_m values may instead reflect the increased cytoplasmic concentrations resulting from the physiological survival strategy of the resistant microbial fraction.

In contrast to C_m, a large decrease of microbial N and P was recorded during summer that supported our hypothesis. Cytoplasmic osmolytes used by bacteria to withstand drought stress are amino compounds (Csonka 1989), while fungi use polyols that do not contain N nor P (Witteveen & Visser 1995). We can speculate whether the observed decrease in N_m and the change in the microbial C/N ratio could indicate that during the summer drought microbial community composition shifted to a higher abundance of fungi (Schimel et al. 2007).

Increased soil microbial N and P were observed during the wet seasons (spring and autumn). In a similar seasonal study, Nielsen et al. (2009) observed that the higher water content increased the accessibility of nutrients and enhanced microbial growth, and consequently N and P immobilization in the microbial biomass. In addition root exudates, root decay and fresh litter shed by the winter-deciduous *Q. canariensis* could constitute an important input of easily decomposable organic substrate for soil microorganism growth (Gallardo & Merino 1993; Joergensen et al. 1994). Both mechanisms could account for the increased microbial nutrient immobilization.

There were also seasonal changes in the concentration of available inorganic nutrients that could be related to variations in temperature, moisture and quality of organic matter, which control microbial processes such as mineralization and immobilisation (Gallardo & Merino 1992; Schmidt et al. 1999). Despite the differences

in soil water availability in the two forest sites due to distinct soil texture and water holding capacity, common patterns were found for inorganic nutrients dynamics: High levels of available N and P, probably resulting from net mineralization and liberation, were recorded during Spring 2007 when conditions were favourable for microbial growth and activity (Figure 2). During summer Nm and Pm decreased, but there is no evidence of increasing available nutrients thus it is possible that inorganic nutrients resulted from microorganisms decay may have been taken up by plants. In Autumn and Spring 2008, N and P were immobilized in the growing microbial biomass. From the synchrony between the available nutrients temporal patterns and microbial biomass dynamics we could infer that in these forests microbes might not be competing with plants for soil resources, but instead could be covering plants nutrient demand. However this hypothesis remains to be critically tested.

Microbial biomass is related to forest site conditions

In a previous study carried out in the same forest sites Quilchano & Marañón (2002) observed lower enzymatic (dehydrogenase) activity in the Forest site. In accordance, our results indicated that microbial C was higher in the Woodland, particularly in soils of *Q. canariensis*, than in the Forest. This difference was consistent across seasons suggesting that the driving mechanism of this variation was not affected by seasonal changes in the environmental conditions. Both sites differ in several physiographical aspects that could contribute to their distinct soil microbial properties; however we suggest that the higher clay content in the Woodland soils may be among the most important factors accounting for this difference since clay has a higher capacity to adsorb nutrients and organic matter. It also reduces decomposition rates, buffers pH changes, provides microorganisms shelter against microbivores and increases the soil

water holding capacity, all of which promote microbial biomass growth (Oades 1988; Van Veen & Kuikman 1990; Wardle 1992).

Season determines canopy cover type effect on microbial biomass

Canopy cover type accounted for main differences in soil microbial parameters within the oak forests; although those patterns were only apparent under favourable environmental conditions, once the main constraining factor (water stress) disappeared. The influence of seasonal conditions on the effect of vegetation on microbial C, N and P has been also detected in other studies (Goberna et al. 2007; Malchair & Carnol 2009). In addition, the effect of canopy cover type was mostly observed in the upper soil layer, while conditions were more homogeneous in the subsurface soil suggesting a plant cover effect on soil probably through differences in litter fall quantity and quality (Augusto et al. 2002). Billore et al. (1995) found a strong positive correlation between microbial and root biomasses, thus vegetation could have also controlled soil microbial processes through differences in the root systems. Nevertheless, not all the variability observed among microhabitats should be attributed the canopy cover type but to other belowground characteristics (e.g. soil depth) that could in turn be the underlying reason for the distinct cover type.

Soils underneath *Q. canariensis* and shrub sustained a higher microbial C and microbial N than soils beneath *Q. suber*. Differences in the litter quality among the vegetation types (lower N content and higher C/N ratio in *Q. suber*; Aponte et al. in press) would have induced a distinct soil organic matter content, total soil nitrogen and C/N ratio and subsequently affected soil microbial C and N (Kara et al. 2008; Rinnan et al. 2008; Smolander & Kitunen 2002). Soils in the forest gaps had the lowest microbial values compared to the other studied microsites. These soils had a higher proportion of

nitrogen (Cm/Nm: 7.6) but a lower fraction of phosphorus (Cm/Pm: 93.8), which could be interpreted as a P limitation (Cleveland & Liptzin 2007). The lack of canopy cover in the forest gaps reduced litter input (0.09kg/m²). The small inputs of P could constrain microbial growth and activity (García et al. 2002; Goberna et al. 2007) and may also increase exposure of this soil to rapid shifts in soil temperature and moisture. In contrast to the Woodland, no differences were found in the microbial components between the soils beneath the two *Quercus* species in the Forest. The vegetation structure of this site, where oak trees had a higher density (Table 1) and formed a closed canopy, promoted higher homogeneity of the litter layer that was reflected in the soil chemical and microbiological properties (Quilchano et al. 2008).

Microbial properties and soil depth

In the studied forest sites all measured microbial constituents, as well as most of the studied soil variables which may influence the microbial pool (soil moisture, SOM, total N, C and P), were higher in the surface soil than in the subsurface soil. A similar pattern has been reported by other authors who also detected a decline in microbial activity with soil depth (Ross et al. 1996). Two main mechanisms could be driving this pattern: first a decrease in the quality and quantity of substrate; the subsurface soil contained less organic matter and probably had a higher fraction of recalcitrant compounds resulted from an advanced decomposition (Gaudinski et al. 2000). Second, the lower moisture content of the subsurface soil could impede the diffusion of the scarce substrate through the disconnected water pockets and limit its supply to the isolated microbial populations (Xiang et al. 2008). Both mechanisms may act simultaneously inducing microbial starvation and limiting microbial population growth in the subsurface soil (Fontaine et al. 2007).

Microbial ratios increased with soil depth in our soils. Contradictory patterns of change in Cm/Nm have been found in other studies. For example Ross *et al.* (1996) observed that Cm/Nm declined with depth in forest soils, whereas Raubuch & Joergensen (1996) detected only a small difference in the microbial C-to-N ratios in the organic layer (6.0) and in mineral soil (7.1). Variations in the C/N ratio are commonly related to shifts in microbial community composition (bacteria vs. fungi), since the fungi have higher carbon: element ratios (C:N: 5-17; N:P=15) than the bacteria (C:N: 6.5 N:P =7) (Cleveland & Liptzin 2007). In his work Fierer et al.(2003) observed that decreasing substrate and moisture availability along the soil depth profile induced changes in microbial community composition and lead to a community dominated by drought and starvation-tolerant organisms.

Conclusions

- In the studied Mediterranean forests soil microbial biomass was affected by season, vegetation cover type and structure, and soil depth.

- Seasonal changes in microbial nutrient content were observed for N and P, which had higher values during the wet seasons (spring and autumn), unlike microbial C.

- Differences in the soil microbial properties between forest sites or among canopy cover types were found in spring but not in summer.

- Microbial C, N and P significantly decreased from surface to subsurface soil, in every season and forest site

The conjoint study of the effects of season, vegetation cover type and structure and soil depth on microbial biomass in two forest sites has shown the existence of relevant seasonal interactions between most of these factors.

The typical seasonal pattern of Mediterranean climate strongly determines the soil moisture regime, affects microbial growth and conditions the influence of other biotic factors on microbial biomass, playing an important role in the nutrient release-immobilization cycles and in the nutrient availability for plants in these forests.

Overall, this study provides valuable information on soil microbial seasonal dynamics in Mediterranean forests that may contribute to enhance our understanding on how climate change could affect to microbial control on nutrient availability.

Acknowledgements

We thank the Consejería de Medio Ambiente (Andalusian Government) and Marco Antonio Tena, then Director of Los Alcornocales Natural Park, for the facilities and support to carry out our field work. We are grateful to Eduardo Gutiérrez, Carlos Ros and Susana Hito for field and lab assistance and to Jorge Castro for introducing us to soil microbial ecology. This study was supported by a FPI-MEC grant to CA, by the Spanish MEC projects Dinamed (CGL2005-5830-C03-01), and Interbos (CGL2008-4503-C03-01), the Andalusian GESBOME Project (RNM 1890), and the European FEDER funds. This research is part of the Globimed (www.globimed.net) network in forest ecology.

526 Appendix 1. Characteristics of the surface (0-8 cm) and subsurface (8-16 cm) soil beneath the studied vegetation cover types in the two
527 forest sites. Data represent mean values and standard error. Letters indicate differences between groups $p < 0.05$ after fdr correction.

	Organic Matter ⁽¹⁾ (%)	Total C (%)	Total N (%)	Total P (ppm)	Ct/Nt	NH ₄ -N (ppm)	NO ₃ -N (ppm)	Available P (ppm)	Clay ⁽²⁾ (%)	Sand ⁽²⁾ (%)	Litter mass ⁽³⁾ (kg m ⁻²)	Litter thickness ⁽³⁾ (cm)
Superficial soil												
Woodland												
<i>Q. canariensis</i>	14.93 (0.51) a	4.36 (0.19) a	0.43 (0.02) a	411.9 (9.5) a	10.67 (0.50) a	9.87 (1.24) a	2.56 (0.28) a	3.17 (0.31) ac	32.7 (2.1) a	46.4 (2.5) a	0.87 (0.07) ab	4.25 (0.26) ab
<i>Q. suber</i>	12.39 (0.37) bc	4.42 (0.18) a	0.33 (0.01) b	332.6 (8.5) b	13.84 (0.43) b	9.05 (0.76) a	2.66 (0.27) a	3.07 (0.27) ac	24.8 (2.4) ab	55.2 (4.1) ab	1 (0.15) b	2.95 (0.40) c
Shrub	14.15 (0.58) ab	4.37 (0.18) a	0.40 (0.02) a	322.1 (10.1) b	12.04 (0.66) ac	9.09 (0.85) a	2.95 (0.30) a	2.56 (0.20) ab	30.1 (3.3) a	46 (4.7) a	0.63 (0.04) a	5 (0.44) a
Grass	11.40 (0.50) c	4.12 (0.13) a	0.31 (0.01) bc	282.0 (9.3) c	14.11 (0.45) bd	5.7 (0.60) b	2.6 (0.28) a	2.31 (0.24) b	30.8 (3.5) a	47.4 (5.0) a	0.04 (0.01) c	0.5 (0.18) d
Forest												
<i>Q. canariensis</i>	12.51 (0.79) c	3.95 (0.20) a	0.32 (0.02) bc	297.8 (10.4) bc	13.44 (0.70) bc	4.79 (0.38) b	2.05 (0.13) a	3.68 (0.24) ac	13.1 (1.3) bc	67.8 (1.5) bc	1.69 (0.10) d	4.5 (0.27) ab
<i>Q. suber</i>	10.81 (0.44) c	4.28 (0.14) a	0.28 (0.01) c	299.1 (8.9) bc	16.02 (0.51) bd	4.26 (0.29) b	1.46 (0.14) b	2.92 (0.17) ac	19.2 (2.2) c	63.4 (2.5) c	1.69 (0.13) d	3.53 (0.33) bc
Subsuperficial soil												
Woodland												
<i>Q. canariensis</i>	9.17 (0.37) a	3.47 (0.13) a	0.24 (0.01) a	324.4 (11.4) a	15.09 (0.33) a	5.72 (0.73) a	2.02 (0.27) ab	2.03 (0.14) a	na	na	-	-
<i>Q. suber</i>	7.08 (0.35) b	2.87 (0.11) b	0.17 (0.01) b	228.2 (11.9) b	17.19 (0.38) a	5.8 (0.51) a	1.73 (0.21) ac	1.98 (0.14) a	na	na	-	-
Shrub	9.32 (0.44) a	3.57 (0.16) a	0.25 (0.01) a	317.3 (13.3) a	15.34 (0.52) a	6.62 (0.71) a	2.13 (0.22) a	1.46 (0.12) b	na	na	-	-
Grass	8.32 (0.29) a	3.13 (0.11) ab	0.21 (0.01) ac	281.1 (8.6) c	15.44 (0.48) a	6.01 (0.50) a	2.54 (0.28) a	1.82 (0.18) ab	na	na	-	-
Forest												
<i>Q. canariensis</i>	8.94 (0.66) a	3.24 (0.17) ab	0.22 (0.01) a	230.7 (8.7) b	15.63 (0.71) a	3.58 (0.40) b	1.35 (0.22) bd	2.64 (0.21) c	na	na	-	-
<i>Q. suber</i>	7.30 (0.29) b	3.05 (0.11) ab	0.19 (0.01) bc	209.4 (8.1) b	17.03 (0.42) a	3.41 (0.36) b	1.17 (0.14) cd	1.93 (0.13) a	na	na	-	-
All average	10.53 (0.18)	3.73 (0.05)	0.28 (0.01)	294.7 (3.7)	14.7 (0.2)	7.41 (0.36)	2.1 (0.07)	2.47 (0.06)	25.1 (1.4)	54.35 (1.8)	1.29 (0.08)	3.76 (0.18)

(1). OM measured by loss on ignition

(2) Textural variables were determined only for 0-25cm soil depth, na mean non available data

(3) Litter variables were measured on the surface of each sample point

References

- Anderson JPE & Domsch KH (1980) Quantities of Plant Nutrients in the Microbial Biomass of Selected Soils. *Soil Sci* 130: 211-216
- Aponte C, García LV, Marañón T & Gardes M (2010) Indirect host effect on ectomycorrhizal fungi: Leaf fall and litter quality explain changes in fungal communities on the roots of co-occurring Mediterranean oaks. *Soil Biol Biochem* (in press). DOI:10.1016/j.soilbio.2010.01.014
- Augusto L, Ranger J, Binkley D & Rothe A (2002) Impact of several common tree species of European temperate forests on soil fertility. *Ann For Sci* 59: 233-253
- Balser TC & Firestone MK (2005) Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* 73: 395-415
- Bates BC, Kundzewicz ZW, Wu S & Palutikof JP (Eds) (2008) *Climate Change and Water*. Technical Paper of the Intergovernmental Panel on Climate Change,. IPCC Secretariat, Geneva
- Billore SK, Ohsawa M, Numata M & Okano S (1995) Microbial biomass nitrogen pool in soils from a warm temperate grassland, and from deciduous and evergreen forests in Chiba, central Japan. *Biol Fertil Soils* 19: 124-128
- Bohlen PJ, Groffman PM, Driscoll CT, Fahey TJ & Siccama TG (2001) Plant-soil-microbial interactions in a northern hardwood forest. *Ecology* 82: 965-978
- Bray RH & Kurtz LT (1945) Determination of total, organic and available forms of phosphorous in soils. *Soil Sci* 59: 39-45

551 Brookes PC, Landman A, Pruden G & Jenkinson DS (1985) Chloroform fumigation and
 552 the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass
 553 nitrogen in soil. *Soil Biol Biochem* 17: 837-842

554 Brookes PC, Powlson DS & Jenkinson DS (1982) Measurement of microbial phosphorus in
 555 soil. *Soil Biol Biochem* 14: 319-329

556 Cleveland CC & Liptzin D (2007) C:N:P stoichiometry in soil: Is there a "Redfield ratio"
 557 for the microbial biomass? *Biogeochemistry* 85: 235-252

558 Crenshaw CL, Lauber C, Sinsabaugh RL & Staveland LK (2008) Fungal control of nitrous
 559 oxide production in semiarid grassland. *Biogeochemistry* 87: 17-27

560 Csonka LN (1989) Physiological and genetic responses of bacteria to osmotic stress.
 561 *Microbiological Reviews* 53: 121-147

562 Díaz-Raviña M, Acea MJ & Carballas T (1993) Seasonal fluctuations in microbial
 563 populations and available nutrients in forest soils. *Biol Fertil Soils* 16: 205-210

564 Fierer N, Schimel JP & Holden PA (2003) Variations in microbial community composition
 565 through two soil depth profiles. *Soil Biol Biochem* 35: 167-176

566 Fisk MC & Fahey TJ (2001) Microbial biomass and nitrogen cycling responses to
 567 fertilization and litter removal in young northern hardwood forests. *Biogeochemistry* 53:
 568 201-223

569 Fontaine S, Barot S, Barre P, Bdioui N, Mary B & Rumpel C (2007) Stability of organic
 570 carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450: 277-U210

571 Gallardo A, Covelo F, Morillas L & Delgado M (2009) Ciclos de nutrientes y procesos
 572 edáficos en los ecosistemas terrestres: especificidades del caso mediterráneo y sus
 573 implicaciones para las relaciones suelo-planta. *Ecosistemas* 18: 4-19

574 Gallardo A & Merino J (1992) Nitrogen immobilization in leaf litter at two Mediterranean
575 ecosystems of SW Spain. *Biogeochemistry* 15: 213-228

576 Gallardo A & Merino J (1993) Leaf Decomposition in Two Mediterranean Ecosystems of
577 Southwest Spain: Influence of Substrate Quality. *Ecology* 74: 152-161

578 Gallardo A, Rodríguez-Saucedo JJ, Covelo F & Fernández-Alés R (2000) Soil nitrogen
579 heterogeneity in a Dehesa ecosystem. *Plant Soil* 222: 71-82

580 García C, Hernandez T, Roldan A & Martin A (2002) Effect of plant cover decline on
581 chemical and microbiological parameters under Mediterranean climate. *Soil Biol Biochem*
582 34: 635-642

583 García LV (2003) Controlling the false discovery rate in ecological research. *Trends Ecol*
584 *Evol* 18: 553-554

585 Gaudinski JB, Trumbore SE, Davidson EA & Zheng S (2000) Soil carbon cycling in a
586 temperate forest: radiocarbon-based estimates of residence times, sequestration rates and
587 partitioning of fluxes. *Biogeochemistry* 51: 33-69

588 Goberna M, Pascual JA, García C & Sánchez J (2007) Do plant clumps constitute microbial
589 hotspots in semiarid Mediterranean patchy landscapes? *Soil Biol Biochem* 39: 1047-1054

590 Hassink J (1994) Effect of soil texture on the size of the microbial biomass and on the
591 amount of c and n mineralized per unit of microbial biomass in dutch grassland soils. *Soil*
592 *Biol Biochem* 26: 1573-1581

593 Hernández T, García C & Reinhardt I (1997) Short-term effect of wildfire on the chemical,
594 biochemical and microbiological properties of Mediterranean pine forest soils. *Biol Fertil*
595 *Soils* 25: 109-116

596 Jenkinson D & Ladd J (1981) Microbial biomass in soil: measurement and turnover. In: E.
 597 A. Paul and J. N. Ladd (ed.) (Ed) Soil biochemistry pp p. 415–471). Marcel Dekker, Inc., .
 598 New York
 599 Joergensen RG, Kübler H, Meyer B & Wolters V (1995) Microbial biomass phosphorus in
 600 soils of beech (*Fagus sylvatica* L.) forests. *Biol Fertil Soils* 19: 215-219
 601 Joergensen RG, Meyer B & Mueller T (1994) Time-course of the soil microbial biomass
 602 under wheat - a one-year field-study. *Soil Biol Biochem* 26: 987-994
 603 Joffre R, Rambal S & Romane F (1996) Local variations of ecosystem functions in
 604 Mediterranean evergreen oak woodland. *Ann. For. Sci.* 53: 561-570
 605 Jonasson S, Michelsen A & Schmidt IK (1999) Coupling of nutrient cycling and carbon
 606 dynamics in the Arctic, integration of soil microbial and plant processes. *Appl Soil Ecol* 11:
 607 135-146
 608 Jonasson S, Michelsen A, Schmidt IK, Nielsen EV & Callaghan TV (1996) Microbial
 609 biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and
 610 sugar: Implications for plant nutrient uptake. *Oecologia* 106: 507-515
 611 Kara Ö, Bolat I, Çakiroğlu K & Öztürk M (2008) Plant canopy effects on litter
 612 accumulation and soil microbial biomass in two temperate forests. *Biol Fertil Soils* 45: 193-
 613 198
 614 Killham K, Amato M & Ladd JN (1993) Effect of substrate location in soil and soil pore-
 615 water regime on carbon turnover. *Soil Biol Biochem* 25: 57-62
 616 Kuikman PJ, Van Vuure MMI & Van Veen JA (1989) Effect of soil moisture regime on
 617 predation by protozoa of bacterial biomass and the release of bacterial nitrogen. *Agric*
 618 *Ecosyst Environ* 27: 271-279

619 Ley RE, Williams MW & Schmidt SK (2004) Microbial population dynamics in an
 620 extreme environment: Controlling factors in talus soils at 3750 m in the Colorado Rocky
 621 Mountains. *Biogeochemistry* 68: 313-335
 622 Lipson DA, Schmidt SK & Monson RK (1999) Links between microbial population
 623 dynamics and nitrogen availability in an alpine ecosystem. *Ecology* 80: 1623-1631
 624 Malchair S & Carnol M (2009) Microbial biomass and C and N transformations in forest
 625 floors under European beech, sessile oak, Norway spruce and Douglas-fir at four temperate
 626 forest sites. *Soil Biol Biochem* 41: 831-839
 627 Miller AE, Schimel JP, Sickman JO, Skeen K, Meixner T & Melack JM (2009) Seasonal
 628 variation in nitrogen uptake and turnover in two high-elevation soils: mineralization
 629 responses are site-dependent. *Biogeochemistry* 93: 253-270
 630 Mlambo D, Mwenje E & Nyathi P (2007) Effects of tree cover and season on soil nitrogen
 631 dynamics and microbial biomass in an African savanna woodland dominated by
 632 *Colophospermum mopane*. *Journal of Tropical Ecology* 23: 437-448
 633 Myers RT, Zak DR, White DC & Peacock A (2001) Landscape-Level Patterns of Microbial
 634 Community Composition and Substrate Use in Upland Forest Ecosystems. *Soil Sci Soc Am*
 635 *J* 65: 359-367
 636 Nielsen PL, Andresen LC, Michelsen A, Schmidt IK & Kongstad J (2009) Seasonal
 637 variations and effects of nutrient applications on N and P and microbial biomass under two
 638 temperate heathland plants. *Appl Soil Ecol* 42: 279-287
 639 Oades J (1988) The retention of organic matter in soils. *Biogeochemistry* 5: 35-70
 640 Ojeda F, Marañón T & Arroyo J (2000) Plant diversity patterns in the Aljibe Mountains (S.
 641 Spain): a comprehensive account. *Biodivers Conserv* 9: 1323-1343

642 Pérez-Ramos IM, Zavala MA, Marañón T, Díaz-Villa MD & Valladares F (2008)
 643 Dynamics of understorey herbaceous plant diversity following shrub clearing of cork oak
 644 forests: A five-year study. *For Ecol Manage* 255: 3242-3253
 645 Quilchano C & Marañón T (2002) Dehydrogenase activity in Mediterranean forest soils.
 646 *Biol Fertil Soils* 35: 102
 647 Quilchano C, Marañón T, Pérez-Ramos I, Noejovich L, Valladares F & Zavala M (2008)
 648 Patterns and ecological consequences of abiotic heterogeneity in managed cork oak forests
 649 of Southern Spain. *Ecol Res* 23: 127-139
 650 Raubuch M & Joergensen RG (2002) C and net N mineralisation in a coniferous forest soil:
 651 the contribution of the temporal variability of microbial biomass C and N. *Soil Biol*
 652 *Biochem* 34: 841-849
 653 Rinnan R, Michelsen A & Jonasson S (2008) Effects of litter addition and warming on soil
 654 carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. *Appl Soil*
 655 *Ecol* 39: 271-281
 656 Ross DJ, Tate KR & Feltham CW (1996) Microbial biomass, and C and N mineralization,
 657 in litter and mineral soil of adjacent montane ecosystems in a southern beech (*Nothofagus*)
 658 forest and a tussock grassland. *Soil Biol Biochem* 28: 1613-1620
 659 Rutigliano FA, D'Ascoli R & Virzo De Santo A (2004) Soil microbial metabolism and
 660 nutrient status in a Mediterranean area as affected by plant cover. *Soil Biol Biochem* 36:
 661 1719-1729
 662 Schade JD & Hobbie SE (2005) Spatial and temporal variation in islands of fertility in the
 663 Sonoran Desert. *Biogeochemistry* 73: 541-553
 664 Schimel J, Balser TC & Wallenstein M (2007) Microbial stress-response physiology and its
 665 implications for ecosystem function. *Ecology* 88: 1386-1394

666 Schimel JP, Scott WJ & Killham K (1989) Changes in Cytoplasmic Carbon and Nitrogen
 667 Pools in a Soil Bacterium and a Fungus in Response to Salt Stress. *Appl Environ Microbiol*
 668 55: 1635-1637

669 Schmidt IK, Jonasson S & Michelsen A (1999) Mineralization and microbial
 670 immobilization of N and P in arctic soils in relation to season, temperature and nutrient
 671 amendment. *Appl Soil Ecol* 11: 147-160

672 Schmidt SK, Costello EK, Nemergut DR, Cleveland CC, Reed SC, Weintraub MN, Meyer
 673 AF & Martin AM (2007) Biogeochemical consequences of rapid microbial turnover and
 674 seasonal succession in soil. *Ecology* 88: 1379-1385

675 Singh JS, Raghubanshi AS, Singh RS & Srivastava SC (1989) Microbial biomass acts as a
 676 source of plant nutrients in dry tropical forest and savanna. *Nature* 338: 499-500

677 Smolander A & Kitunen V (2002) Soil microbial activities and characteristics of dissolved
 678 organic C and N in relation to tree species. *Soil Biol Biochem* 34: 651-660

679 Sparks DL (1996) *Methods of Soil Analysis. Part 3. Chemical Methods* Soil Science
 680 Society of America and American Society of Agronomy, Madison, Wisconsin, USA

681 Urbieto I, Zavala M & Marañón T (2008) Human and non-human determinants of forest
 682 composition in southern Spain: evidence of shifts toward cork oak dominance due to
 683 management over the past century. *J Biogeogr* 35: 1688-1700

684 Soil Survey Staff (2006) *Keys to Soil Taxonomy*, 10th ed. USDA-Natural Resources
 685 Conservation Service, Washington, DC.

686 Van Veen J & Kuikman P (1990) Soil structural aspects of decomposition of organic matter
 687 by micro-organisms. *Biogeochemistry* 11: 213-233

688 Vance ED, Brookes PC & Jenkinson DS (1987) An extraction method for measuring soil
 689 microbial biomass C. *Soil Biol Biochem* 19: 703-707

690 Wardle DA (1992) A comparative assesment of factors which influence microbial biomass
691 carbon and nitrogen levels in soil. Biol Rev 67: 321-358
692 Witteveen CFB & Visser J (1995) Polyol pools in *Aspergillus niger*. FEMS Microbiol Lett
693 134: 57-62
694 Xiang SR, Doyle A, Holden PA & Schimel JP (2008) Drying and rewetting effects on C
695 and N mineralization and microbial activity in surface and subsurface California grassland
696 soils. Soil Biol Biochem 40: 2281-2289
697
698

FIGURE LEGEND

Figure 1. Location of the study area and the two forest sites in the Iberian Peninsula

Figure 2. Soil water content estimated gravimetrically at the two forest sites (F-Forest; W-Woodland) and the four studied vegetation cover types (Qc- *Q. canariensis*; Qs- *Q. suber*; S- shrub; G- grass) at each sampling season.

Figure 3. Microbial C, N and P estimated in the soils under the different types of vegetation cover (*Q. canariensis* (Qc), *Q. suber* (Qs), shrub (S) and grass cover (G)) in the two studied forest sites (Forest and Woodland) across the four sampling seasons (Spring 07, Summer 07, Autumn 07, Spring 08). Data is presented for the two soil depths, surface soil (0-8 cm) and subsurface soil (8-16 cm). Letters indicate differences between groups for each season ($p < 0.05$ after FDR corrections). Bars represent standard error of the mean.

Figure 4. Microbial ratios -Cm/Nm, Cm/Pm and Nm/Pm- for each season (Spring 07, Summer 07, Autumn 07, Spring 08), and vegetation cover type (*Q. canariensis* (Qc), *Q. suber* (Qs), shrub (S) and grass cover (G)) in the two forest sites (Forest and Woodland). Data is presented for the two soil depths (0-8cm and 8-16cm). Letters indicate differences between groups for each season ($p < 0.05$ after FDR corrections). Bars represent standard error of the mean

Figure 5. Microbial C, N and P, K₂SO₄-extractable dissolved organic carbon (DOC) and nitrogen (DON), inorganic nitrogen (extractable ammonium and nitrate; Ni) and available inorganic phosphorus (Pi) estimated in the surface soil (0-8 cm) beneath *Quercus* cover in the Woodland and the Forest site across the sampling seasons.

721 TABLES

722 **Table 1.** Climate and vegetation structure of the studied forest sites. Sources are the
 723 AEMET for climate and Pérez-Ramos et al. (2008) for vegetation.

724

	Forest	Woodland
Mean Rainfall (mm)		
Spring	216.8	258.9
Summer	21.1	28.0
Autum	262.3	319.2
Winter	472.2	526.4
Total	972.3	1132.4
Mean Temperature (°C)		
Annual	16.6	15.5
Minimum	4.1	1.8
Maximum	23.4	23.6
Vegetation structure		
Density of trees (stems.ha ⁻¹)	768.8	218.8
Density of arborescent shrubs (stems.ha ⁻¹)	256.3	450.0
Basal area (m ² ha ⁻¹)	47.0	22.1
Leaf area index (m ² m ⁻²)	2.26	1.84

Table 2. Microbial P, C and N (mean and standard error) and their ratios. Seasonal and soil depth values are averaged for forest sites (Woodland and Forest), and canopy cover type (under *Q. canariensis*, *Q. suber*, shrub and grass).

	Pmic		Cmic		Nmic		Cm/Nm	Cm/Pm	Nm/Pm	Cm/Ct	Nm/Nt	Pm/Pt
	(µg g ⁻¹)		(µg g ⁻¹)		(µg g ⁻¹)					(%)	(%)	(%)
Woodland												
<i>Q. canariensis</i>	25.67	(2.03)	1119.62	(70.32)	147.68	(10.85)	9.60	62.29	6.88	2.93	4.13	6.8
<i>Q. suber</i>	22.46	(2.03)	825.87	(48.72)	108.31	(8.32)	10.17	63.48	6.58	2.29	4.18	7.9
Shrub	21.32	(1.86)	966.27	(53.81)	138.58	(10.21)	8.97	79.61	8.97	2.50	4.11	7.0
Grass	12.18	(0.88)	772.75	(38.87)	107.86	(6.11)	7.57	93.80	12.19	2.11	4.14	4.4
Forest												
<i>Q. canariensis</i>	16.88	(1.38)	596.81	(32.39)	95.91	(5.55)	6.98	77.43	10.37	1.71	3.70	6.2
<i>Q. suber</i>	16.26	(1.43)	638.29	(37.64)	89.44	(4.99)	8.03	92.26	11.06	1.67	3.94	6.3
Woodland	20.39	(0.92)	921.13	(27.98)	125.61	(4.62)	9.08	74.81	8.66	2.46	4.14	6.5
Forest	16.57	(0.99)	617.55	(24.80)	92.68	(3.73)	7.51	84.85	10.71	1.69	3.82	6.2
All average	19.14	(0.70)	819.93	(21.41)	114.63	(3.39)	8.56	78.12	9.34	2.20	4.03	6.4

735 **Table 3.** Repeated measures ANOVA for microbial C, N and P of surface soil (0-8 cm) and subsurface soil (8-16 cm) from the two studied
736 forest sites measured across four seasons. F and p-values for between effects (forest site and canopy cover type), within effect (season) and
737 two way interactions are presented. Significant univariate results for each sampling season are indicated as *(0.05>p≥0.01); **(
738 0.01>p≥0.001); ***(p<0.001).

739

	Cm						Nm						Pm					
	F	p	Sp07	Su07	A07	Sp08	F	p	Sp07	Su07	A07	Sp08	F	p	Sp07	Su07	A07	Sp08
Surface soil																		
Forest site	40.45	0.000	**	**	***	***	15.55	0.000	**	**		***	18.127	0.000	***		*	***
Season	0.99	0.399					36.82	0.000					32.455	0.000				
Season* Forest site	6.56	0.000					10.74	0.000					24.233	0.000				
Vegetation cover	4.21	0.042	**				10.74	0.001	**			**	0.552	0.459				
Forest site* Vegetation cover	13.74	0.000	**			***	3.79	0.053	*				0.503	0.479				
Subsurface soil																		
Forest site	17.25	0.000	*		*	**	1.000	0.324	***		***	**	11.500	0.002	***		***	
Season	2.05	0.110					34.594	0.000					22.780	0.000				
Season* Forest site	1.88	0.137					28.598	0.000					22.646	0.000				
Vegetation cover	3.99	0.048					3.158	0.078	*				3.051	0.083				
Forest site* Vegetation cover	2.77	0.098	*				1.434	0.233					0.149	0.700				

Forest site: Woodland and Forest

Vegetation cover type: *Q. canariensis* and *Q. suber*

Season: spring 07, summer 07, autumn 07, spring 08









